MINIATURIZING AND AUTOMATING CELL VIABILITY AND REPORTER ASSAYS FOR HIGH-THROUGHPUT AND ULTRAHIGH-THROUGHPUT SCREENING

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Screening facilities require simple solutions for measuring and correlating a variety of parameters of cellular processes. Robust assays and high-precision, low-volume, non-contact pipetting systems are now providing this capability. We have demonstrated the scalability of the CellTiter-Glo®, Caspase-Glo™ 3/7, and Chroma-Luc™/Chroma-Glo™ Technologies for high-throughput and ultrahigh-throughput cell-based screening in low-volume 384- and 1536-well formats.

Introduction

The ability to measure and correlate one or more responses within a cellular process is an essential part of life science research. As high-throughput screening has become more common in research facilities, the need for higher density, lower volume assays is increasing. Today, the trend toward fully automated, miniaturized assays continues through the use of low-volume 384 (LV384)- and 1536-well formats. Such formats require assays that have no human interventions during the screening process and that deliver consistent results in single-microliter volumes.

The short incubation times and extended luminescent signal half-lives of each chemistry allow continuous and batch-mode plate processing.

Here we demonstrate the capability of Promega cell-based assays to meet the demands of automated high-throughput or ultrahigh-throughput users. Assays were performed using the CellTiter-Glo® Luminescent Cell Viability Assay[a,b], a cell viability assay based on the quantitation of ATP in metabolically active cells; the Caspase-Glo™ 3/7 Assay[a,b], a caspase assay that measures the activity of caspase-3 and -7; and the Chroma-Glo™ Luciferase Assay System[b-e] in combination with the Chroma-Luc™ Vectors[c,e,f,g], a dual-color luciferase methodology that can quantify and correlate multiple parameters associated with a cellular process. These assays were performed in LV384- and 1536-well formats. Cells, cell lysates, and reagents were dispensed using one of two commercially available low-volume, non-contact pipetting systems, in volumes ranging from 10µl to 500nl. Assay plates were read using a number of PMT- or CCD-based HTS plate readers commonly found in screening facilities.

HTS-Adaptable Chemistries

The CellTiter-Glo®, Caspase-Glo™ 3/7, and Chroma-Luc™/Chroma-Glo™ Technologies represent chemistries that are ideally suited for high-throughput and ultrahigh-throughput screening. Each requires adding only a single reagent to the well, eliminating the need for time-consuming rinsing of pipetting tips. The homogeneous format also allows users to miniaturize the assay, provided that the one-to-one ratio of reagent to culture medium is preserved. The short incubation times and extended luminescent signal half-lives of each chemistry allow continuous and batch-mode plate processing.

Table 1. Percent CV Values for the CellTiter-Glo®, Caspase-Glo™ 3/7 and Chroma-Luc™/Chroma-Glo™ Assays Performed in LV384- and 1536-Well Formats.

Chemistry	Assay Format	Assay Volume	Cell Number (per well)	%C.V.
CellTiter-Glo™Assay	LV384	20µl	5,000	2.23
		10µl	2,500	3.21
		5µl	1,250	2.71
		2µl	500	2.53
	1536	8µl	2,000	2.42
		5µl	1,250	2.73
		2µl	500	3.90
		1µl	250	3.26
		0.5µl	125	5.12
Caspase-Glo™3/7 Assay	LV384	20µl	5,000	4.98
		10µl	2,500	2.82
		5µl	1,250	8.95
		2µl	500	9.72
	1536	8µl	2,000	5.27
		5µl	1,250	5.07
		2µl	500	7.78
		1µl	250	6.12
		0.5µl	125	8.53

Chroma-Luc™/ Chroma-Glo™ Assay

Assay Format	Assay Volume	Lysate	Cell Equiv. Conc.	% C.V.
1536-well	8µl	Red	2,000	9.32
		Green	2,000	9.21

Cell concentrations are based upon 250 cells/µl standard. CellTiter-Glo® and Caspase-Glo™ 3/7 Assays were run on the Equator™ NS-808 Pipetting System and read using the PHERAstar instrument. Chroma-Luc™/Chroma-Glo™ Assays were run on the FlexDrop™ IV workstation and imaged using the ViewLux™ instrument. Chroma-Glo™ Assays were performed as described later in the text of this article for the Z′-factor experiments.

Automated Non-Contact High-Throughput Liquid Dispensing

The use of high-density, low-volume plate formats to screen compound libraries containing millions of test compounds has created the need for pipetting systems capable of rapidly

Table 2. Linearity and Limit of Detection for LV384- and 1536-Well Format CellTiter-Glo® and Caspase-Glo™ 3/7 Assays.

Assay Format	Assay Volume	Chemistry	Cell Range Tested (Cells/Well)	Cell Strain	Linearity r^2 value	Limit of Detection (Cells/Well)
LV384	20µl	CellTiter-Glo® Assay	0–5,000	Jurkat	0.9987	10
		Caspase-Glo™ 3/7 Assay	0–10,000	Jurkat	0.9944	156
	10µl	CellTiter-Glo® Assay	0–2,500	Jurkat	0.9994	1
		Caspase-Glo™ 3/7 Assay	0–5,000	Jurkat	0.9997	78
	5µl	CellTiter-Glo® Assay	0–2,500	Jurkat	0.997	1
		Caspase-Glo™ 3/7 Assay	0–2,500	Jurkat	0.9975	78
	2µl	CellTiter-Glo® Assay	0–1,000	Jurkat	0.9951	1
		Caspase-Glo™ 3/7 Assay	63–1,000	Jurkat	0.9973	125
1536	8µl	CellTiter-Glo® Assay	0–4,000	Jurkat	0.9974	1
			0–2,000	d293	0.9966	125
		Caspase-Glo™ 3/7 Assay	0–4,000	Jurkat	0.9999	250
			0–1,000	d293	0.9979	63
	5µl	CellTiter-Glo® Assay	0–2,500	Jurkat	0.996	1
			0–1,250	d293	0.9954	78
		Caspase-Glo™ 3/7 Assay	0–2,500	Jurkat	0.9996	156
			0–625	d293	0.9986	39
	2µl	CellTiter-Glo® Assay	0–1,000	Jurkat	0.993	1
			0–500	d293	0.9942	31
		Caspase-Glo™ 3/7 Assay	0–1,000	Jurkat	0.9998	63
			1–250	d293	0.9934	16
	1µl	CellTiter-Glo® Assay	0–500	Jurkat	0.9893	1
			0–250	d293	0.9903	16
		Caspase-Glo™ 3/7 Assay	0–500	Jurkat	0.9991	31
			4–125	d293	0.998	31
	0.5µl	CellTiter-Glo® Assay	0–250	Jurkat	0.9901	1
		Caspase-Glo™ 3/7 Assay	0–250	Jurkat	0.9981	31

CellTiter-Glo® and Caspase-Glo™ 3/7 Assays were run on the Equator™ NS-808 and read using the PHERAstar instrument. Apoptosis was induced in Jurkat cells as described in Technical Bulletin #TB323. d293 cells were treated in 10% Triton® X-100 before adding Caspase-Glo™ Reagent and assaying as described in TB323.

delivering reagents in a consistent fashion across smaller volume ranges. To meet these demands, screening facilities have turned to non-contact liquid handling systems that can deliver reagents to a plate in seconds and that can repeat this process for hundreds of plates without cross-contamination between wells. In order to test Promega cell-based chemistries using this type of dispensing technology, the Perkin Elmer FlexDrop™ IV Reagent Dispenser or the Deerac Fluidics Equator™ NS-808 Eight-Tip Pipetting System was used to pipette cells, cell lysates and reagents to the assay plates. Table 1 shows that %CVs for all assays performed in LV384- and 1536-well formats were less than 10% in volumes as low as 500nl.

HTS/uHTS Microplate Analysis

The process of screening compound libraries in high-density plate formats can generate hundreds of assay plates requiring analysis. High-end HTS and uHTS microplate readers and imagers have been developed to simply and efficiently accomplish this task. To qualify Promega cell-based chemistries on instruments commonly found in screening facilities, we ran assays using our CellTiter-Glo®, Caspase-Glo™ 3/7, and Chroma-Luc™/Chroma-Glo™ Technologies in LV384- and 1536-well formats. Assay plates were then analyzed using either the BMG LABTECH PHERAstar Microplate Reader, the Molecular Devices Analyst® GT Multimode Reader, or the Perkin Elmer ViewLux™ ultraHTS Microplate Imager.

Testing and Results

CellTiter-Glo® and Caspase-Glo™ 3/7 Assays

Each chemistry was tested in LV384- and 1536-well formats according to its performance criteria. CellTiter-Glo® and Caspase-Glo™ 3/7 Assays were performed to analyze sensitivity and linearity across a range of cell concentrations in total volumes (cell media + reagent) ranging from 20 to 2µl in LV384-well format and from 8µl to 500nl in 1536-well format (Table 2). Limit of Detection was calculated as the difference between sample averages minus two standard deviations and background plus two standard deviations.

Z′-factor anaylsis was used to characterize the assay variability and dynamic range (Table 3; ref. 1). All assays performed with these two chemistries were run on the Equator™ NS-808 Eight-Tip Pipetting System, and plates were subsequently analyzed using the BMG LABTECH PHERAstar Microplate Reader.

Chroma-Luc™ Reporters and Chroma-Glo™ Reagent

We evaluated the Chroma-Luc™ technology for assay sensitivity and linearity for red and green luciferases, using lysates at various cell equivalent concentrations (Figure 1). Red (CBR*luc*) and green (CBG99*luc*) luciferase lysates were used for these evaluations. The lysates were prepared by adding Glo Lysis Buffer (Cat.# E2661) to CHO cells stably transfected with either CBR*luc* or CBG99*luc* genes. Assays were run using an 8µl total volume in 1536-well format. Limit of Detection was calculated as the difference between sample averages and background plus three standard deviations, as described in Technical Manual #TM062.

Separation of the two luminescent signals was also assessed using various combinations of the red and green luciferase lysates (Figure 2). Lysates dispensed using the PerkinElmer FlexDrop™ IV Reagent Dispenser were dispensed at 100/0, 75/25, 50/50, 25/75, and 0/100 percent concentrations of red/green luciferase lysate, respectively. These plates were analyzed using the Molecular Devices Analyst® GT Multimode Reader or the PerkinElmer ViewLux™ ultraHTS Microplate Imager. Lysates dispensed using the Deerac Fluidics Equator™ NS-808 Eight-Tip Pipetting System were dispensed at 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100 percent concentrations of red/green luciferase lysate, respectively. These plates were analyzed using the BMG LABTECH PHERAstar Microplate Reader.

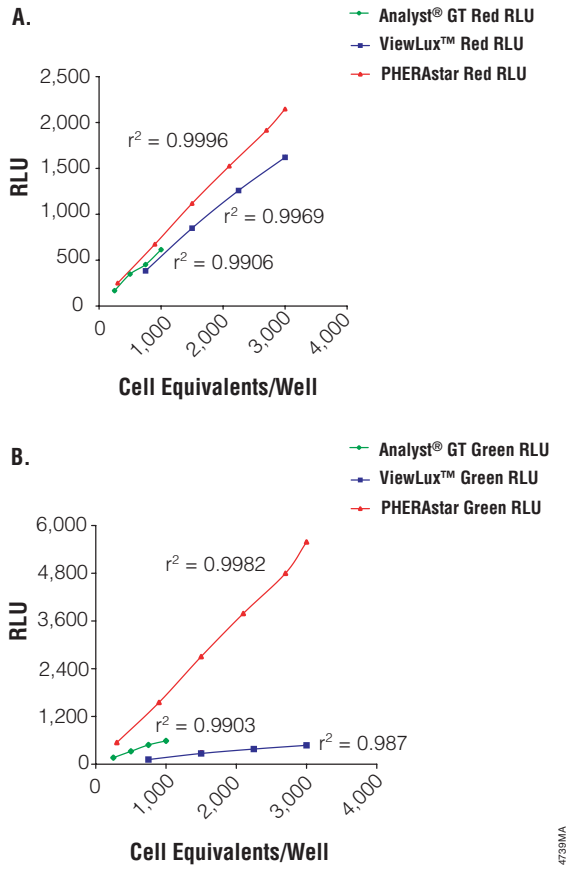

Figure 1. Linearity and sensitivity of Chroma-Glo™ chemistry using red (CBR*luc*; Panel A) and green (CBG99*luc*; Panel B) luciferases. Cell equivalents of 250–1,000 were run on the FlexDrop™ IV and read using the Analyst® GT. Cell equivalents of 750–3,000 were run on the FlexDrop™ IV and read using the ViewLux™ reader. Cell equivalents of 300–3,000 were run on the Equator™ NS-808 and read using the PHERAstar instrument. All assays were performed with an 8µl total volume in 1536-well format.

Table 3. Z´-Factor Values for LV384- and 1536-Well Formats for the CellTiter-Glo® and Caspase-Glo™ 3/7 Assays.

Chemistry	Assay Format	Assay Volume	Z'-Factor Score
CellTiter-Glo® Assay	LV384	20µl	0.85
		10µl	0.77
		5µl	0.68
	1536	8µl	0.84
		5µl	0.85
		2µl	0.82
		1µl	0.70
Caspase-Glo™ 3/7 Assay	LV384	20µl	0.83
		10µl	0.78
		5µl	0.76
	1536	8µl	0.77
		5µl	0.79
		2µl	0.71
		1µl	0.62

Jurkat cells were used for these assays.

Z´-factor analysis was used to describe assay variability and dynamic range (Table 4). Induced and uninduced cells, cotransfected with Chroma-Luc™ Vectors were used in these analyses and to ascertain fold induction values with this chemistry. HEK 293 cells were cotransfected with pCRE-CBG99*luc* and pCRE-CBR*luc* genes. The cells were then treated with either isoproterenol HCl(1µM)/RO(100µM) or RO(100µM) at 24 hours post-transfection. At 3 hours post-induction, medium was removed, and the cells were lysed with 20µl Glo Lysis Buffer and frozen at –80°C. Induced and uninduced lysates were pooled and used for all Z´-Factor and fold-induction determinations. Lysates and reagent were dispensed in 20µl and 10µl total volumes in LV384-well format, or in 8µl, 5µl and 2µl total volumes in 1536-well format. Pipetting lysate and reagent to assay plates was completed using either the PerkinElmer FlexDrop™ IV Reagent Dispenser or the Deerac Fluidics Equator™ NS-808 Eight-Tip Pipetting System. Relative light units were detected using the Molecular Devices Analyst® GT Multimode Reader containing 510–60nm and 610LongPass filters and the PerkinElmer ViewLux™ ultraHTS Microplate Imager containing 540–25nm and 618-8nm filters. Simultaneous detection at both wavelengths was performed with the BMG LABTECH PHERAstar microplate reader containing 537nm short-pass and 610nm long-pass filters.

Table 4. Z´-Factor and Fold Induction Values of LV384 and 1536-Well Format Chroma-Glo™ Assays.

Assay Format	Assay Volume	Cell Equiv. Per Well	Reader	Instrument Filter	Z'-Factor Value	Fold Induction
384	20µl	10,000	Analyst®-GT	Red	0.86	13.08
				Green	0.92	10.01
			ViewLux™	Red	0.89	12.85
				Green	0.90	9.79
			PHERAstar	Red	0.84	14.88
				Green	0.86	13.37
	10µl	5,000	PHERAstar	Red	0.81	13.84
				Green	0.77	13.17
1536	8µl	4,000	ViewLux™	Red	0.77	14.01
				Green	0.75	10.49
			PHERAstar	Red	0.75	12.54
				Green	0.72	7.07
	5µl	2,500	PHERAstar	Red	0.78	15.05
				Green	0.82	12.35
	2µl	1,000	PHERAstar	Red	0.67	14.65
				Green	0.74	12.18

Fold induction was calculated by comparing average values of induced and uninduced pooled lysates using data obtained from the Z´-factor analysis.

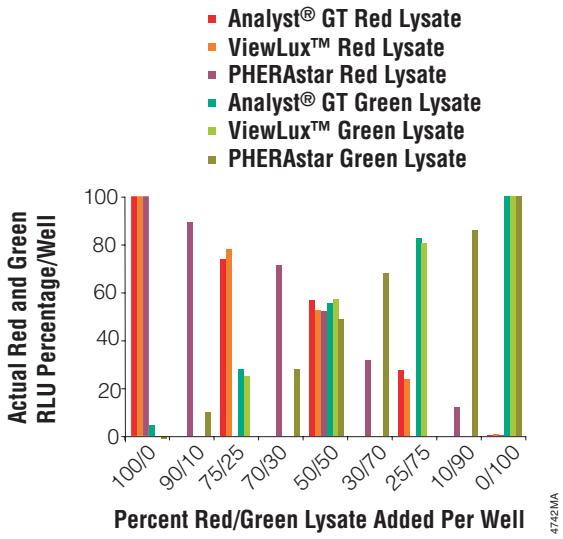

Figure 2. Graph showing comparison of actual separation of red and green luminescent signals to ideal separation percentages. Lysates dispensed using the FlexDrop™ IV and analyzed using either the Analyst® GT or the ViewLux™ readers were dispensed at 100/0, 75/25, 50/50, 25/75, and 0/100 percent concentrations of red and green luciferase lysate, respectively. Lysates dispensed using the Deerac Fluidics Equator™ NS-808 and analyzed using the BMG LABTECH PHERAstar were dispensed at 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100 percent concentrations of red and green luciferase lysate, respectively. Ideal RLU percentages equal the percent of red and green lysate added per well. Assays were performed in 1536-well plates.

Summary

The results shown here illustrate the flexible nature and adaptability of the CellTiter-Glo®, Caspase-Glo™ 3/7, and Chroma-Luc™/Chroma-Glo™ chemistries in the high-throughput and ultrahigh-throughput setting. The experiments performed demonstrate the high degree of linearity across wide ranges of cell and cell lysate concentrations and the extreme sensitivity that each assay is able to achieve in LV384- and 1536-well formats. Excellent Z´-factor scores for all chemistries, as well as fold induction demonstrated with the Chroma-Luc™/Chroma-Glo™ technology, attest to the robustness of each chemistry in the volumes and well formats currently being used in screening facilities.

The single-reagent addition, homogeneous format, short incubation times and extended luminescent signal half-lives make each of these assay chemistries ideally suited for all high-throughput and ultrahigh-throughput screening environments. ■

Reference

1. Zhang, J. *et al.* (1999) *J. Biomol. Screening* **4**, 67–73.

Protocols

Chroma-Glo™ Luciferase Assay System Technical Manual #TM062
(www.promega.com/tbs/tm062/tm062.html)

Chroma-Luc™ Vectors Technical Manual #TM059
(www.promega.com/tbs/tm059/tm059.html)

Caspase-Glo™ 3/7 Assay Technical Bulletin #TB323
(www.promega.com/tbs/tb323/tb323.html)

CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin #TB288
(www.promega.com/tbs/tb288/tb288.html)

Web Sites

www.promega.com/techserv/apps/automat/

www.deerac.com

www.bmglabtech.com

Ordering Information

Product	Size	Cat.#
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573
Caspase-Glo™ 3/7 Assay*	2.5ml	G8090
	10ml	G8091
	100ml	G8092
Chroma-Glo™ Luciferase Assay System	10ml	E4910
	100ml	E4920
	10 × 100ml	E4950
Chroma-Luc™ Vectors		
pCBR-Basic Vector	20µg	E1411
pCBR-Control Vector	20µg	E1421
pCBG68-Basic Vector	20µg	E1431
pCBG68-Control Vector	20µg	E1441
pCBG99-Basic Vector	20µg	E1451
pCBG99-Control Vector	20µg	E1461

*For Laboratory Use.

[a]U.S. Pat. No. 6,602,677, Australian Pat. No. 754312 and other patents pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,073, and 5,700,632.

[c]Certain applications of this product may require licenses from others.

[d]U.S.Pat. Nos. 5,283,179, 5,641,641, 5,650,289, and 5,814,471, Australian Pat. No. 649289 and other patents.

[e]U.S. Pat. Nos. 6,387,675 and 6,552,179, Australian Pat. No. 698424 and other patents pending.

[f]A limited license under Promega's U.S. Pat. Nos. 6,387,675 and 6,552,179, Australian Pat. No. 698424, U.S. Pat. Appln. Ser. No. 09/645,706 and various corresponding patent applications and issued patents is conveyed with the purchase of this product. The terms of the limited license are as follows: For Nonprofit Organization research use only. Specifically, researchers at Nonprofit Organizations may use this product in their research and they may transfer derivatives of the product to colleagues at other Nonprofit Organizations provided that such colleagues agree in writing to be bound by the terms and conditions of this label license. Researchers may not transfer this product or its derivatives to researchers at other organizations, which are not Nonprofit Organizations, without the express written consent of Promega and without those entities having an express license from Promega for the use of the product. Other than as specifically provided here, an authorized transferee of this product shall have no right to transfer this product or its derivatives to any other person or entity. A Nonprofit Organization performing research using this product for a for-profit organization also shall be considered for-profit. For-profit entities purchasing this product shall have six (6) months to evaluate the product in their research. Thereafter, such for-profit entities shall either contact Promega Corporation and take a license for their continued use of the product or destroy the product and all derivatives thereof. With respect to any therapeutic or diagnostic uses by any entity, please contact Promega Corporation for supply and licensing information.

[g]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.